US Environmental Protection Agency
Office of Pesticide Programs

Office of Pesticide Programs
Microbiology Laboratory
Environmental Science Center, Ft. Meade, MD

Standard Operating Procedure for Air/Surface Monitoring of Microbiology Laboratories

SOP Number: QC-02-03

Date Revised: 03-22-07

EPA/OPP MICROBIOLOGY LABORATORY
ESC, Ft. Meade, MD

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Initiated By: _____ Date: ___/___/___

Print Name:_____

Technical Review: _____ Date: ___/___/___

Print Name:_____
Technical Staff

QA Review: _____ Date: ___/___/___

Print Name:_____
QA Officer

Approved By: _____ Date: ___/___/___

Print Name:_____
Branch Chief

Effective Date: ___/___/___

Controlled Copy No.: _____

Withdrawn By: _____ Date: ___/___/___

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1.0 SCOPE AND APPLICATION:

1.1 This SOP describes the method for determining the presence and number of airborne microorganisms that contaminate laboratory surfaces. This procedure is based on aspects of references 15.1 and 15.2. Additional attributes have been added to detect airborne contamination in specific environments.

2.0 DEFINITIONS:

2.1 CFU = Colony Forming Unit

2.2 BSC = Biological Safety Cabinet

2.3 TSA = Tryptic Soy Agar

2.4 TSB = Tryptic Soy Broth

2.5 NB = Nutrient Broth

3.0 HEALTH AND SAFETY: Not applicable

4.0 CAUTIONS: None

5.0 INTERFERENCES:

5.1 Building construction, power outages and equipment maintenance may cause transient aberrant counts (see 14.0). These events should be considered in interpreting results of the air testing and efficacy tests conducted during that time. Note these events in the comments section of the Monitoring Record Form (see 16.1).

5.2 Media must pass sterility assessment in advance of use.

6.0 PERSONNEL QUALIFICATIONS:

6.1 Personnel are required to be knowledgeable of the procedures in this SOP. Documentation of training and familiarization with this SOP can be found in the training file for each employee.

7.0 SPECIAL APPARATUS AND MATERIALS: None

8.0 INSTRUMENT OR METHOD CALIBRATION: Not applicable

9.0 SAMPLE HANDLING AND STORAGE: Not applicable

10.0 PROCEDURE AND ANALYSIS:

10.1 Summary: In this method, a general growth medium/broth is used to monitor the density of airborne microorganisms in the laboratory. The test will be performed on an as needed basis. Petri plates containing TSA (see SOP MB-10, Media and Reagents Used in Efficacy Testing) are placed throughout each laboratory in areas where inoculation, filtering, plating and transfer work is performed. In addition, tubes (38 × 100 mm) containing 20 mL broth (NB or TSB) will also be placed in selective areas. The plates/tubes are exposed to the environment for 15-60 minutes followed by incubation at 36±1°C for 1 to 7 days. Following/during incubation, colonies on the plates are counted and presence/absence of growth in tubes is recorded.

10.2 Label TSA plates with numbers corresponding to the numbered spots on the diagrams for rooms: B206, B204, B202, B203, B205, B207, B201 and B209 (see 16.2). Place plates on each appropriate spot in each laboratory. Place tubes containing broth (NB or TSB) in selective areas.

10.3 Start the timer and remove the covers sequentially at 15-30 second intervals.

10.4 Sequentially replace the covers after 15-60 minutes of exposure time.

10.5 Incubate the plates/tubes at 36±1°C for 1 to 7 days. Plates/tubes may be observed daily for growth.

10.6 Presumptive identification of contaminants may be determined by use of Gram stain and/or Vitek® analysis.

11.0 DATA ANALYSIS/CALCULATIONS:

11.1 Determine the number of CFUs per 15 × 100 mm plate (up to 300 CFU) per 15 minute period (or multiply with the factor if the exposure time is more than 15 minutes, e. g., the number of CFUs be multiplied with 2 if the exposure time is 30 minutes (Ref. 15.2).

12.0 DATA MANAGEMENT/RECORDS MANAGEMENT:

12.1 Data will be recorded promptly, legibly and in indelible ink on the Air Monitoring Record Form (see 16.0). Completed forms are archived in notebooks kept in secured file cabinets in file room D217. Only authorized personnel have access to the secured files. Archived data is subject to OPP's official retention schedule contained in SOP ADM-03, Records and Archives.

13.0 QUALITY CONTROL:

13.1 The OPP Microbiology Laboratory conforms to 40 CFR Part 160, Good Laboratory Practice Standards. Appropriate quality control measures are

integrated into each SOP.

13.2 For quality control purposes, the required information is documented on the appropriate forms (see 16.0).

14.0 NONCONFORMANCE AND CORRECTIVE ACTION:

14.1 If contamination exceeds 15 colonies/plate/15 minute (Ref 15.2) or a laboratory base line on the basis of past historical data (based on review by laboratory management), possible contamination sources will be investigated. In order to minimize or remove the contamination source, the laboratory will undergo a decontamination process that will consist of general cleaning and the application of an antimicrobial product, if necessary, in specific areas. Following this process, the air monitoring procedure will be repeated and operation in a laboratory may be suspended until the problem is resolved.

14.2 If a plate/tube that corresponds to a location inside a BSC demonstrates unacceptable contamination (15 colonies/plate/15 minute or a laboratory base line on the basis of past historical data), the BSC will not be used and the facility maintenance staff will be informed of the situation. If necessary, decontamination procedures (e.g. disinfectant application) will be conducted in the BSC. The monitoring test will be repeated for the affected BSC. The BSC will not be used until the air monitoring indicates acceptable level of microbial counts.

14.3 If any unusual air handling events take place (e.g., significant laboratory air flow changes, construction within the facility), air monitoring may be conducted at any time to verify the quality of the lab's air according to procedures in section 10.0 of this SOP (the test will be conducted only on the affected laboratory).

15.0 REFERENCES:

15.1 Bordner, R.H., Winter, J.A., & Scarpino, P.V., eds. 1978. Microbiological Methods for Monitoring the Environment, Water, and Wastes. EPA 600/8-78-017, Environmental Monitoring & Support Lab., U.S. Environmental Protection Agency, Cincinnati, Ohio.

15.2 Eaton, A.D., Clesceri, L.S., Rice E. W. eds. 2005. Standard Methods for the Examination of Water and Wastewater, 21st Edition. American Public Health Association, American Water Works Association, Water Environment Federation.

16.0 FORMS AND DATA SHEETS:

16.1 Air Monitoring Record Form

16.2 Laboratory Diagrams

Air Monitoring Record Form
OPP Microbiology Laboratory

SOP QC-02 INFORMATION SHEET					Page 1 of 2
Test Performed By: Date/Init.		Incubation Date and Time			
Test Read By: Date/Init.		Start:		Stop:	
Media/Prep. No.:		Media/Prep. No.:			

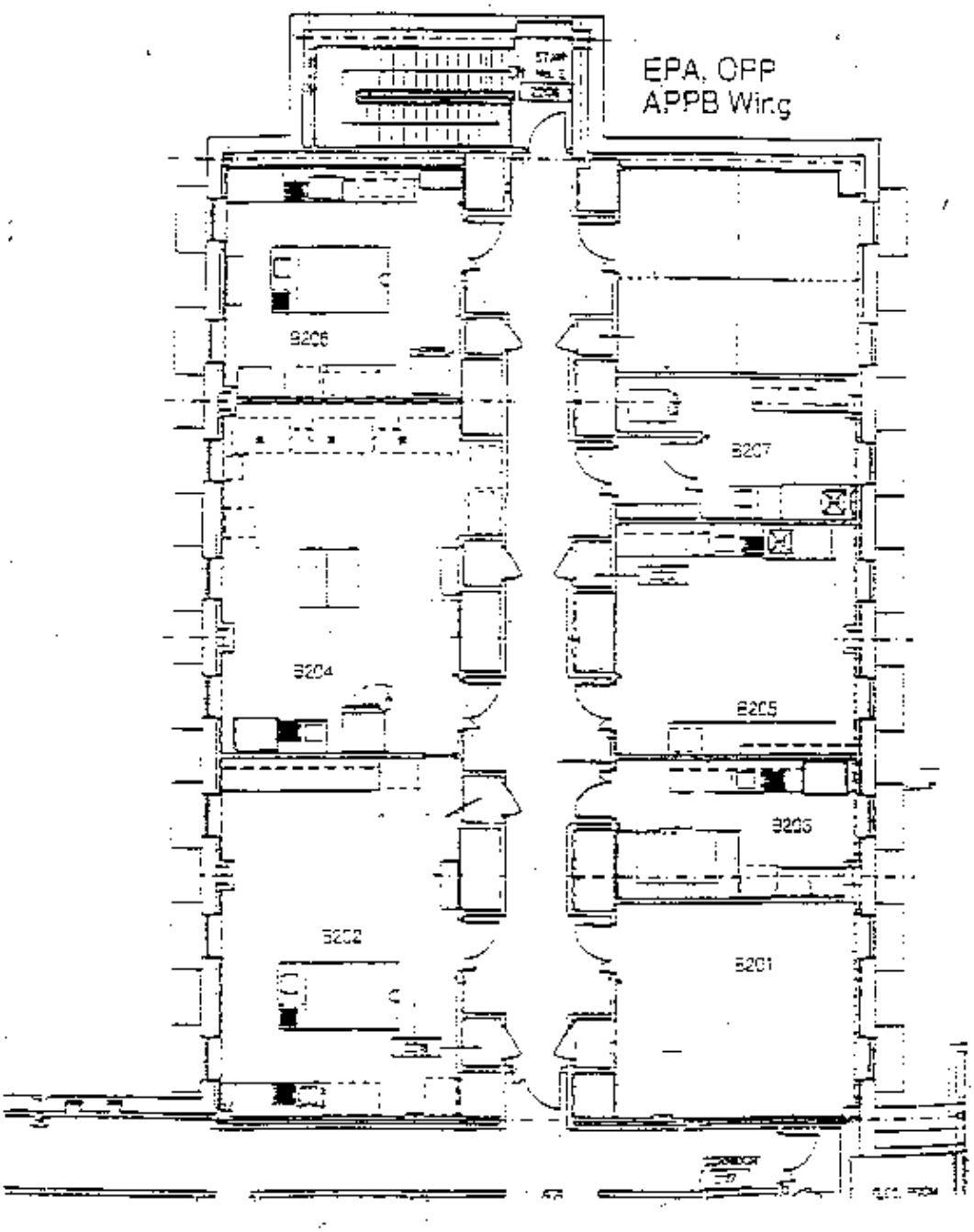
Location	Plate #	CFU/Plate	Tube #	Growth in Broth (+/0)
B206 Glassware & Media Preparation Room	1		1	
	2		2	
	3		3	
	4		4	
	5		5	
	6		6	
	7		7	
	8		8	
B204 Antimicrobial Testing Laboratory	9		9	
	10		10	
	11		11	
	12		12	
	13		13	
	14		14	
	15		15	
B202 Isolation Laboratory	16		16	
	17		17	
	18		18	
	19		19	
	20		20	
	21		21	
	22		22	
	23		23	
	24		24	

Air Monitoring Record Form
OPP Microbiology Laboratory

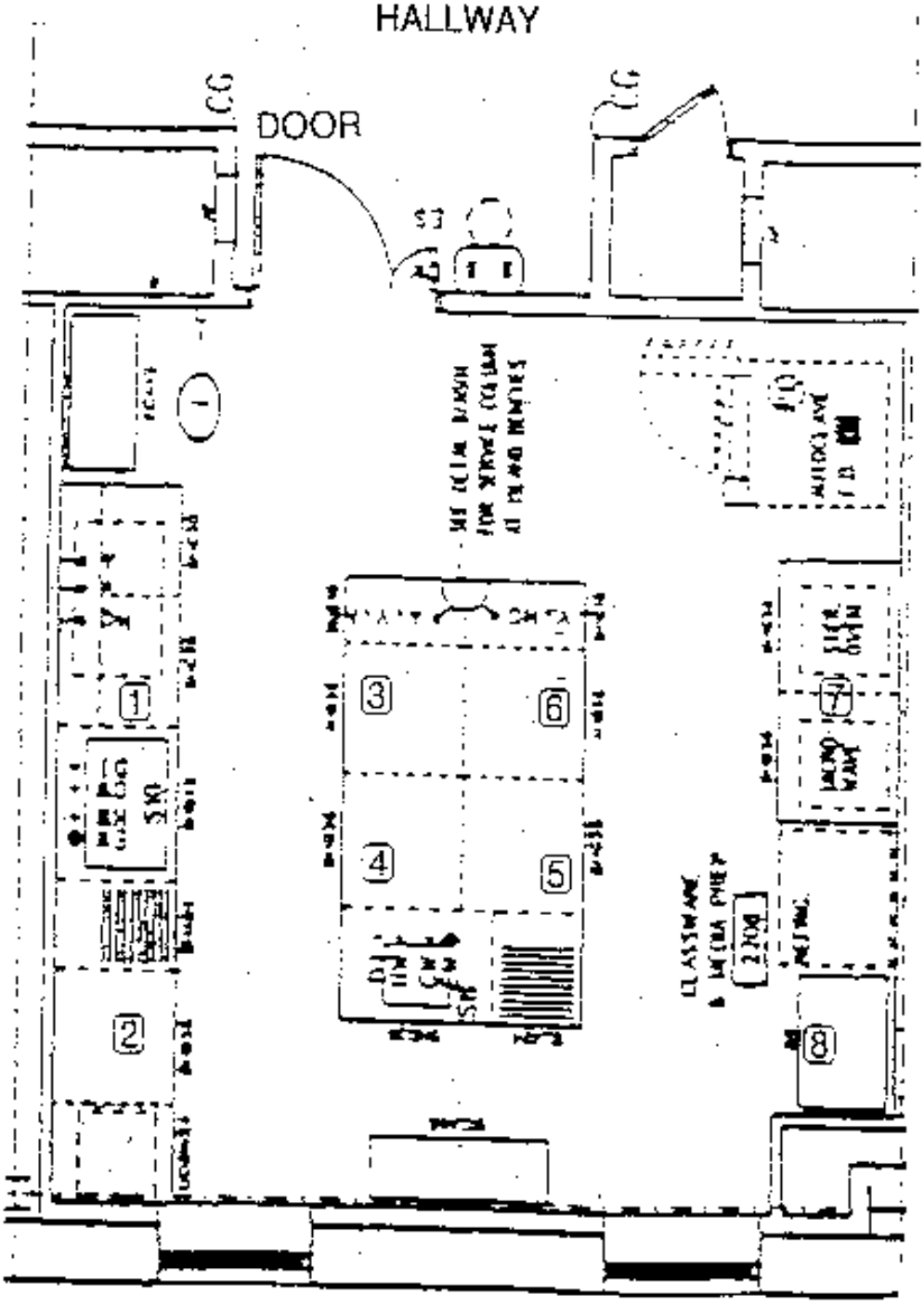
SOP QC-02 INFORMATION SHEET					Page 2 of 2
Test Performed By: Date/Init.		Incubation Date and Time			
Test Read By: Date/Init.		Start:		Stop:	
Media/Prep. No.:		Media/Prep. No.:			

Location	Plate #	CFU/Plate	Tube #	Growth in Broth (+/0)
B203 Incubator Room	25		25	
	26		26	
	27		27	
B205 Special Studies Laboratory	28		28	
	29		29	
	30		30	
	31		31	
	32		32	
B207 Isolation Laboratory	33		33	
	34		34	
	35		35	
	36		36	
	37		37	
	38		38	
	39		39	
B201 Molecular Analysis Laboratory	40		40	
	41		41	
	42		42	
	43		43	
	44		44	
B209 Glassware & Media Preparation Room	45		45	
	46		46	
	47		47	
	48		48	
Unopened Control[1]				
Unopened Control[1]				
Comments				

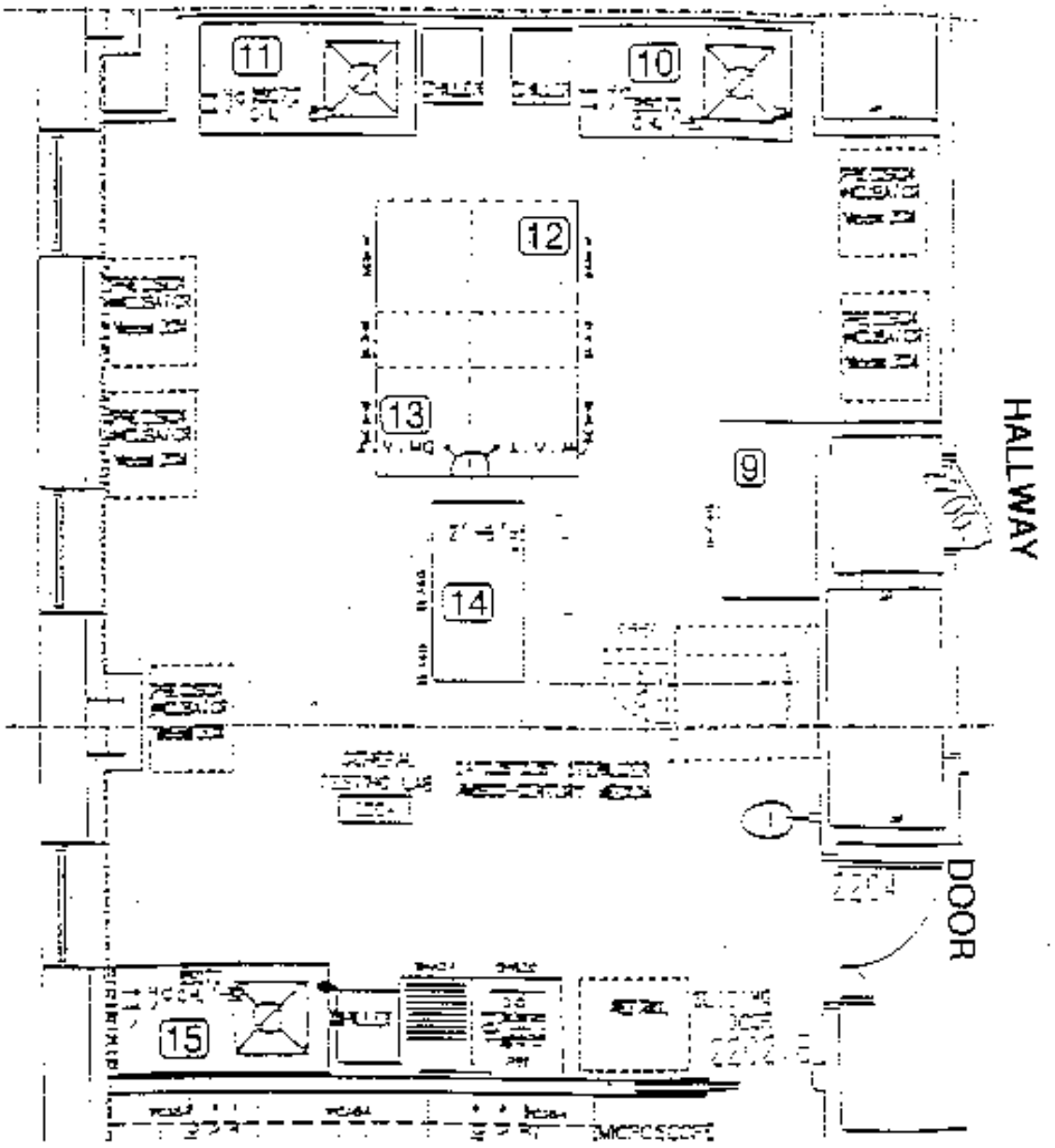
[1]One plate per each batch of different TSA or tube of broth (different prep. no.) should be designated as an unopened control.



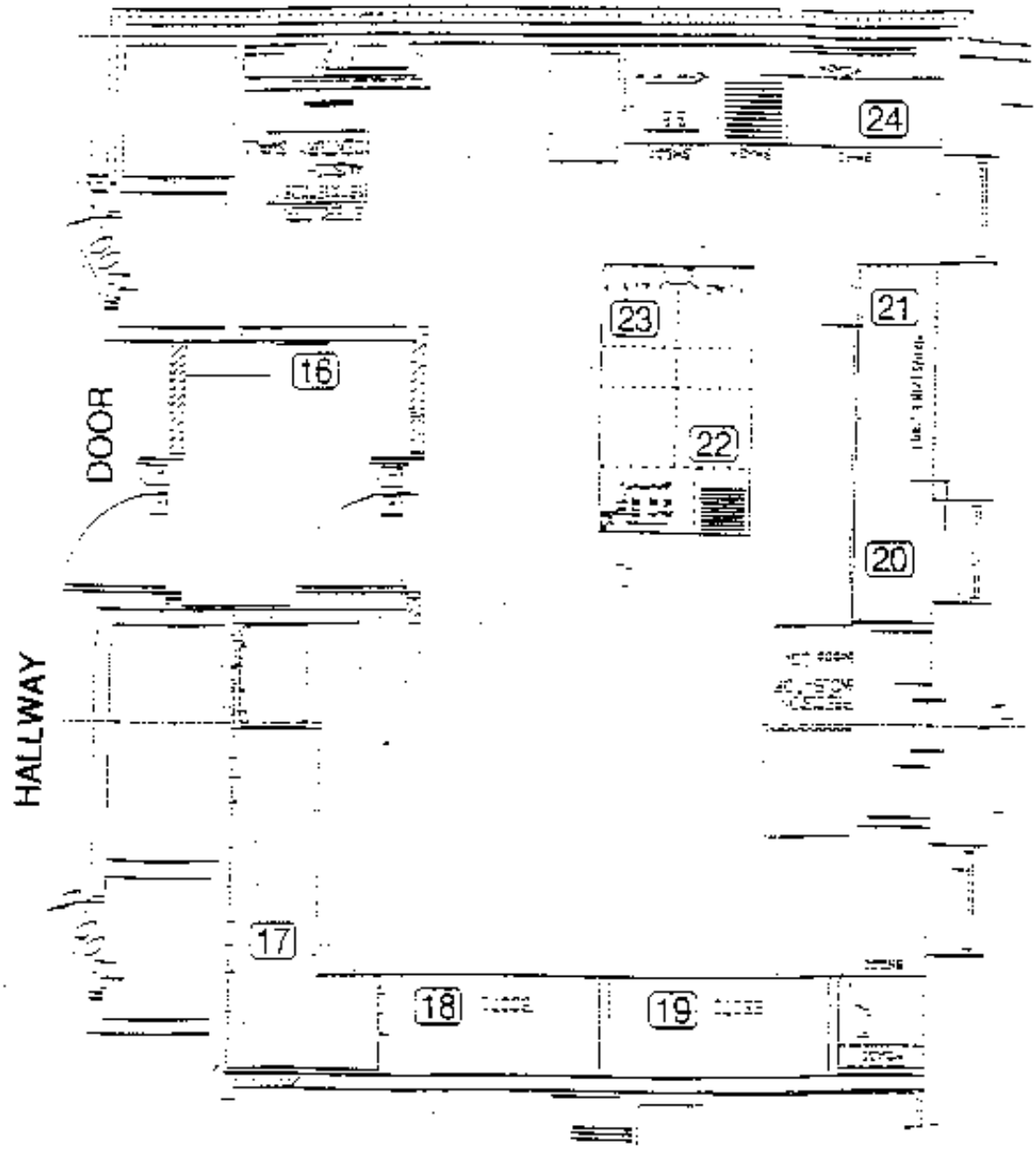
B206 Glassware & Media Preparation Room



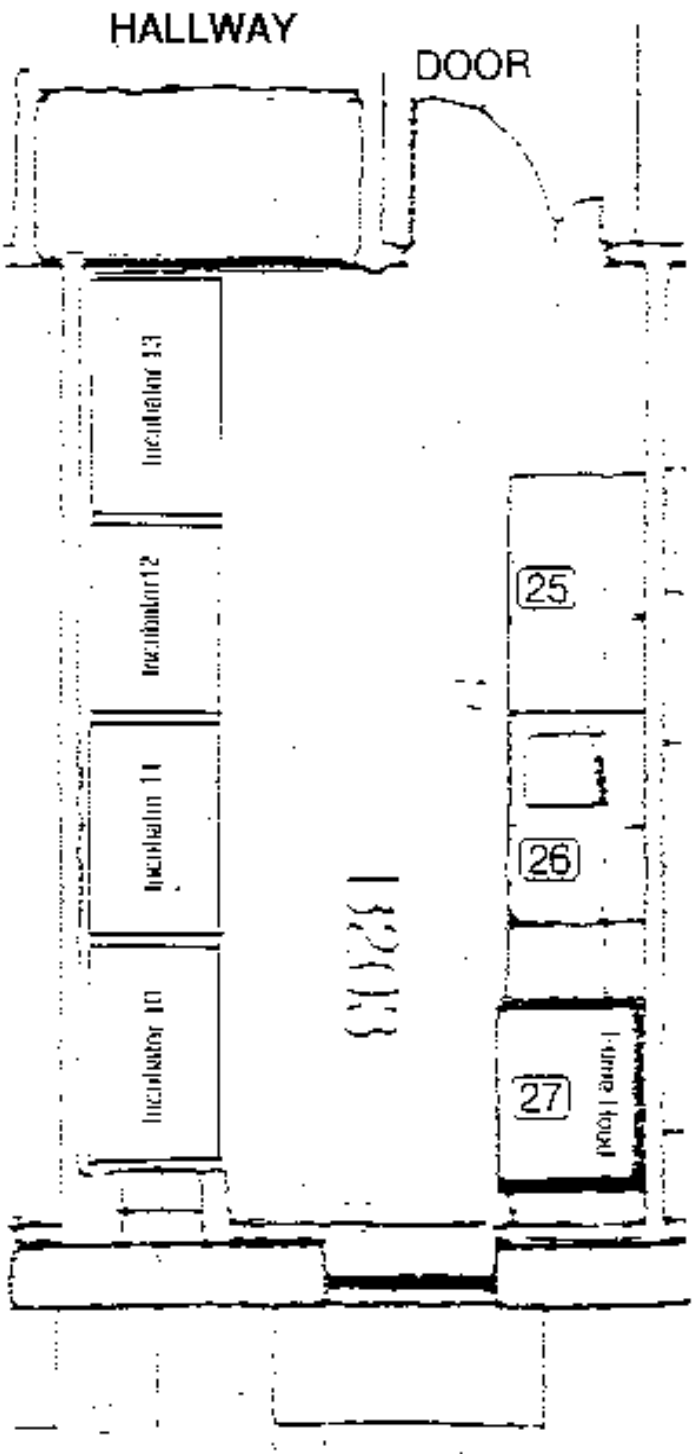
B204 Antimicrobial Testing Laboratory



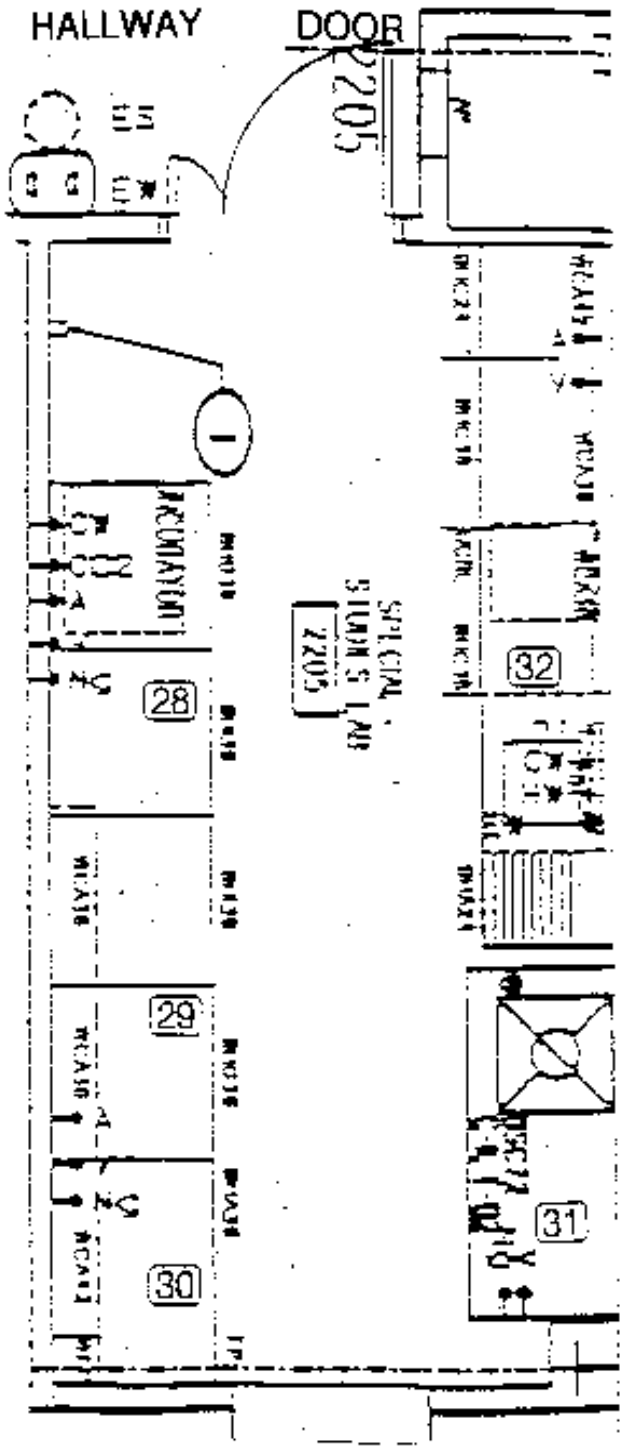
B202 Isolation Laboratory

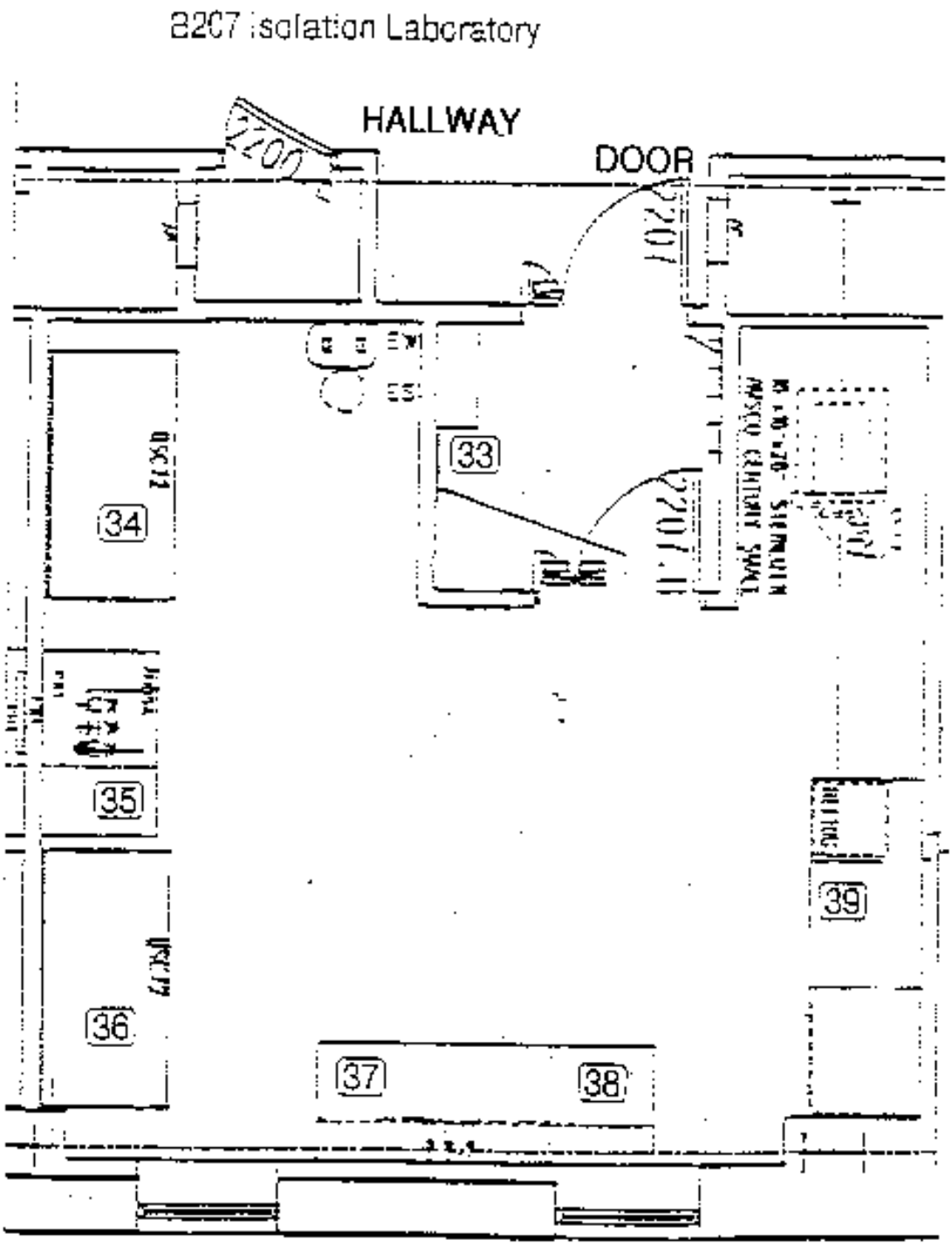


B203 Incubator Room

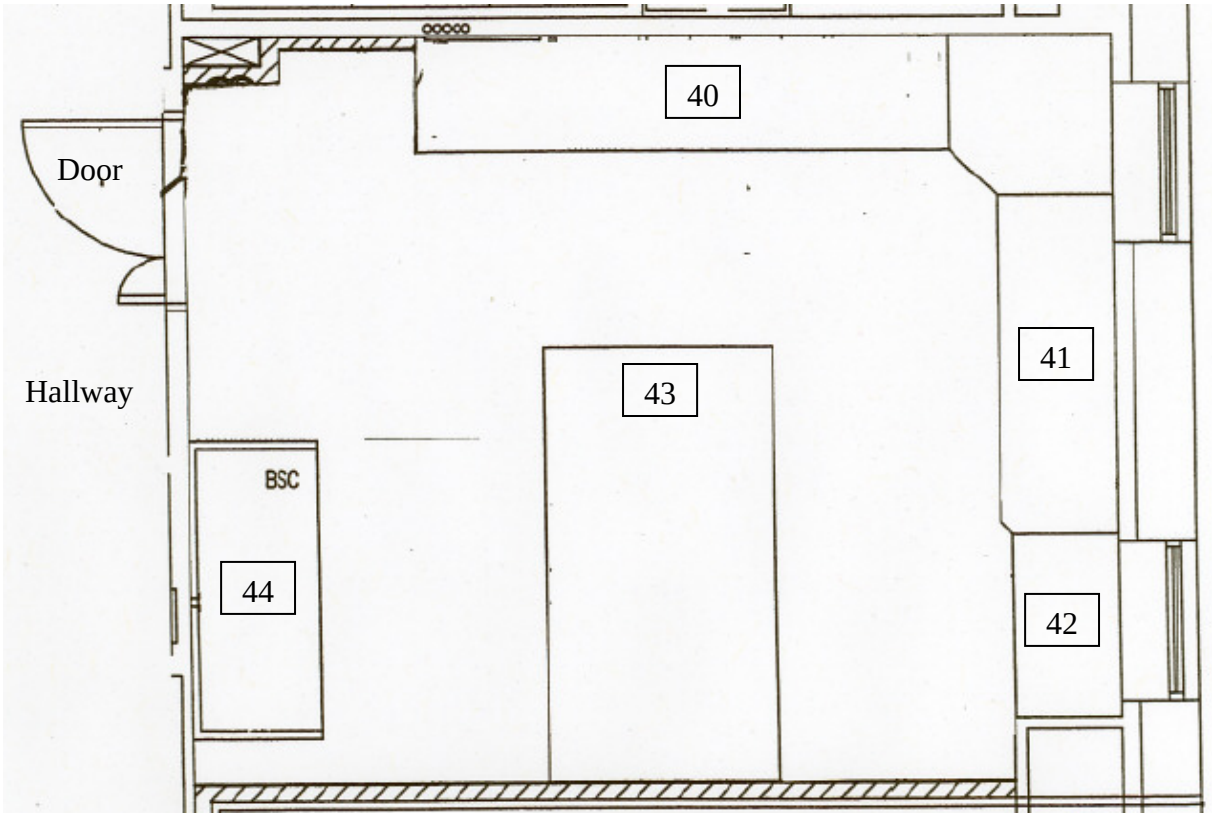


B205 Special Studies Laboratory




2207 Isolation Laboratory
HALLWAY
DOOR
2201
2207
33
34
35
36
37
38
39

B201 Molecular Analysis Laboratory



B209 Glassware & Media Preparation Room Laboratory